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Article

The Environment versus Genetics in Controlling the Contribution of MAP Kinases to Synaptic Plasticity

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Summary

Background: A challenge in biomedical research is to design experimental paradigms that reflect a natural setting. Even when freshly isolated tissues are used, they are almost always derived from animals housed in cages that poorly reflect the animal's native environment. This issue is highlighted by studies on brain function, where mice housed in a more natural "enriched environment" display enhanced learning and memory and delayed onset of symptoms of neurodegenerative diseases compared to mice housed conventionally. How the environment mediates its effects on brain function is poorly understood.
Results: We show that after exposure of adolescent mice to an "enriched environment," the induction of long-term potentiation (LTP), a form of synaptic plasticity that is thought to contribute to learning and memory, involves a novel signal transduction pathway that is nonfunctional in comparable mice housed conventionally. This environmentally gated signaling pathway, which rescues defective LTP induction in adolescent Ras-GRF knockout mice, consists of NMDA glutamate receptor activation of p38, a MAP kinase that does not contribute to LTP in mice housed conventionally. Interestingly, the same exposure to environmental enrichment does not have this effect in adult mice.
Conclusions: This study reveals a new level of cell signaling control whereby environmental factors gate the efficacy of a specific signaling cascade to control how LTP is induced in adolescent animals. The suppression of this gating mechanism in mature animals represents a new form of age-dependent decline in brain plasticity.

Introduction

The proper execution of complex animal functions and their breakdown in disease involves an interaction between genetics and the environment. Although a wealth of information is available on the roles that individual genes play in such processes, how environmental factors contribute is only beginning to be understood. In studies of brain function, comparisons have been made between housing animals in standard laboratory conditions and housing them in an enriched environment (EE), which can include larger cages, various stimulatory objects such as toys of different compositions, shapes, and sizes, and opportunities for voluntary physical activity [1]. EE has been shown to improve learning and memory [2–4], to overcome learning defects caused by genetic alterations in transgenic mice [5], and even to delay the onset of a variety of neurological diseases in animal models including Huntington's and Alzheimer's diseases, epilepsy, and Fragile X syndrome [6–8]. Many changes in the brain have been observed in response to EE, such as enhanced dendritic branching [9, 10], synaptic density [5], neurogenesis [10–13], and expression levels and activities of specific neuronal proteins [14–17] (for review, see [8]). However, little is known about EE-induced alterations of specific signaling pathways that mediate its effects on neuronal function in either normal or diseased states.

Two important cellular functions that may mediate improved learning and memory associated with EE are long-term potentiation (LTP) and long-term depression (LTD) [18–21]. Both are long-lasting changes in synaptic transmission within a synapse in response to a distinct stimulation pattern, with LTP referring to stimulus-induced enhancement of transmission and LTD to stimulus-induced suppression of transmission. Much new information on the biochemical mechanisms underlying these opposing forms of synaptic plasticity has been revealed in the past few years, with some common principles emerging. However, it has also become clear that the mechanisms involved can vary depending upon the specific synapse and circuit studied, the type of stimulation used to induce LTP and LTD, and the developmental stage of the animal being investigated [22]. For example, in many synapses, such as in the CA1 region of the hippocampus, LTP and LTD are mediated in large part by postsynaptic mechanisms involving NMDA-type glutamate receptor (NMDAR)-induced changes in the trafficking of AMPA-type glutamate receptors [23]. LTP increases receptor number on the surface by enhancing their delivery to the plasma membrane, and LTD reduces receptor number by enhancing their removal. However, at this [24] and other synapses, presynaptic mechanisms involving changes in the release of neurotransmitter also have been observed [25].

A variety of intracellular signaling molecules have been implicated in mediating the induction and maintenance of LTP and LTD [18, 26]. Among them, the MAP kinase family members, Erk and p38, have attracted significant attention. LTP induction by a variety of protocols, as well as LTP maintenance, require Erk MAP kinase activation [27]. However, Erk MAP kinase has also been implicated in some forms of LTD induction [28]. In contrast, p38 MAP kinase has been implicated in the generation of LTD, but no reports yet link it to promoting LTP [29–31].

Ras-GRF1 [32] and Ras-GRF2 [33] are both neuronal calcium/calmodulin-regulated exchange factors that

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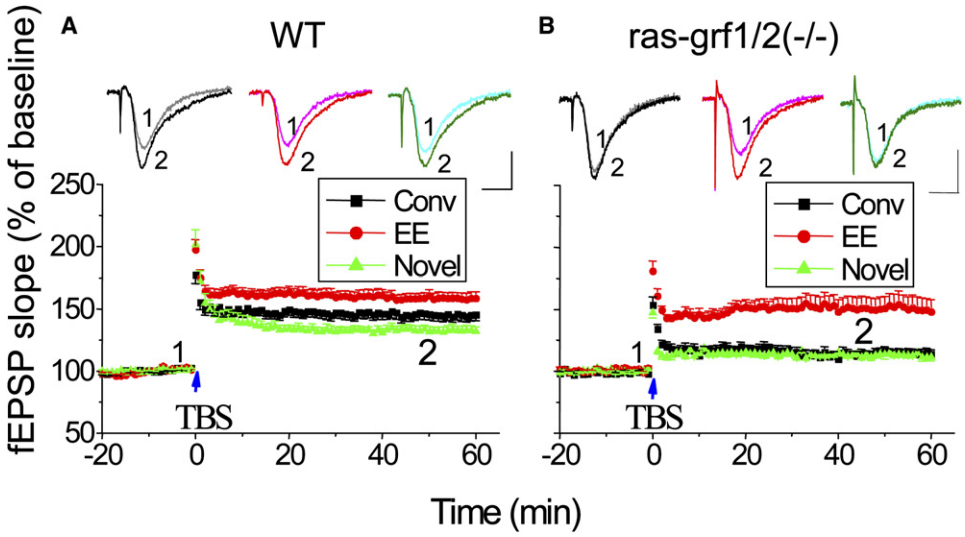

Figure 1. Enriched Environment Rescues Impaired LTP in Young Ras-GRF Knockout Mice

1-month-old wild-type (WT) (A) and Ras-GRF1/Ras-GRF2 double knockout mice (B) mice were exposed to a conventional environment (Conv) or an enriched environment (EE) for 2 weeks. Some mice were exposed to EE only once for 2 hr right before sacrifice (Novel). Theta burst stimulation (TBS, 15 bursts of 4 pulses at 100 Hz, interburst interval 200 ms) was used to induce LTP in the CA1 region of the hippocampal slices from these mice. The recorded fEPSP slope is expressed as percent of baseline ± SEM, and data were pooled from each group.

(A) Conv cages, black boxes, n = 16 slices/10 mice; EE, red circles, n = 18 slices/11 mice; single exposure to the enriched environment, Novel, green triangle, n = 7 slices/4 mice.

(B) Conv, black boxes, n = 12 slices/8 mice; EE, red circles, n = 9 slices/6 mice, Novel, green triangles, n = 8 slices/5 mice.

Insets: representative fEPSP recorded before (1) and after (2) TBS in wild-type and *grf1/grf2*$^{-/-}$ slices. Horizontal calibration line, 10 ms; vertical line, 1 mV.

have the capacity to activate Ras and Rac GTPases [34–37]. By studying defects in signaling pathways and synaptic plasticity in the CA1 region of the hippocampus of single and double Ras-GRF knockout mice, we recently discovered that Ras-GRF1 plays a role predominantly in coupling calcium flux through NMDARs to activation of p38 MAP kinase and LTD. In contrast, Ras-GRF2 plays a role predominantly in coupling NMDARs to activation of Erk MAP kinase and LTP. Ras-GRF protein expression is developmentally regulated [38, 39], and thus the contribution of either Ras-GRF protein to synaptic plasticity begins only after ~postnatal day 20 in mice [39–41].

Importantly, almost all of these mechanistic studies on LTP and LTD have been performed on samples from animals housed in a conventional environment, a condition that can be considered "impoverished" when compared to a more "enriched" natural animal environment. In this study, we detect the presence of a new signaling component of LTP induction in adolescent, but not mature, mice exposed to an enriched environment. This signaling cascade involves p38 MAP kinase, which regulates LTD but not LTP in mice housed conventionally. Thus, the environment has the age-dependent power to determine how synaptic plasticity is induced in mice by gating the flow through a specific MAP kinase-signaling cascade.

Results

An Enriched Environment Rescues Defective LTP Induction Present in Ras-GRF Knockout Mice

We recently showed that Ras-GRF2 contributes to long-term potentiation (LTP) by coupling NMDA receptors (NMDARs) to Erk MAP kinase activation, while Ras-GRF1 contributes to long-term depression by coupling NMDARs to p38 MAP kinase [41]. Thus, double Ras-GRF knockout mice display defects in both of these mechanisms of synaptic plasticity. However, these experiments, as well as the majority of previous experiments aimed at revealing biochemical pathways that regulate synaptic plasticity, were performed with mice housed in a conventional laboratory setting. This involves groups of up to five mice in small cages with only plain bedding, food, and water. Compared to conditions experienced by animals in the wild, such conventional laboratory conditions can be considered impoverished [1]. Thus, to determine the functional significance of Ras-GRF proteins in mice housed more naturally, we investigated synaptic plasticity properties of hippocampal brain slices from Ras-GRF knockout mice derived from mice exposed to an enriched environment (EE). The protocol for EE [16, 42] involved a larger cage (45 × 30 × 25 cm) containing plastic play tubes, cardboard boxes, and nesting material that were changed or rearranged every other day to provide novel stimulation and a running wheel for voluntary exercise. Mice exploring the enriched cage for 6 hr/day for 14 days served as the enriched environment (EE) group, while mice exploring the enriched cage once for only 2 hr before sacrifice served as novelty control group. 1-month-old mice were chosen for this study because the expression of Ras-GRF proteins is developmentally regulated [38] such that at this age, Ras-GRF proteins have just recently begun to contribute to synaptic plasticity [39–41].

Figure 1A shows theta burst-induced LTP in the CA1 region of the hippocampal slices from wild-type animals exposed to either a conventional environment (Conv), an enriched environment for 6 hr/day for 2 weeks (EE), or

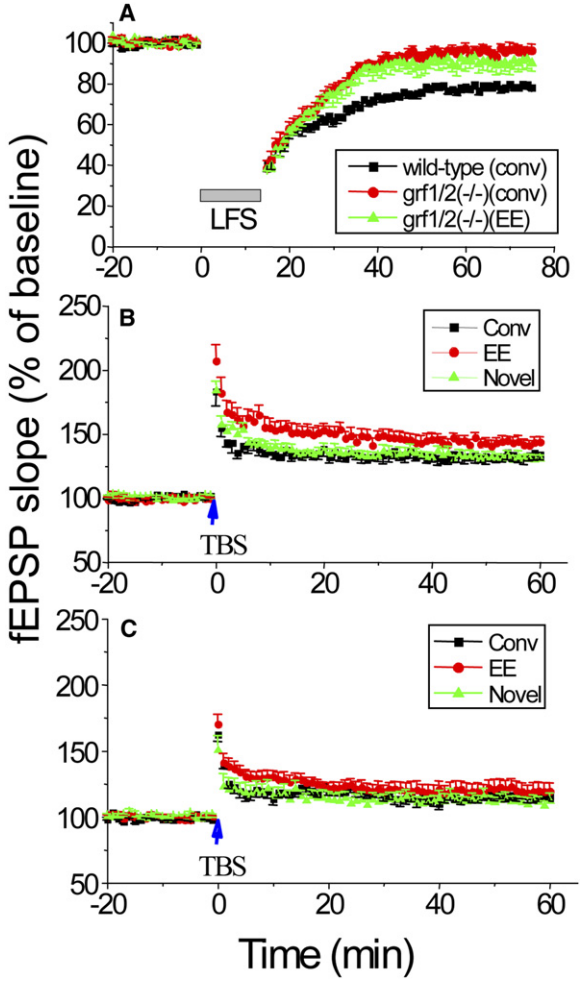

Figure 2. Enriched Environment Fails to Rescue Impaired LTD in Young Mice or LTP in Mature Ras-GRF Knockout Mice

(A) Low-frequency stimulation (LFS) (900 pulses at 1 Hz) was used to induce LTD in hippocampal brain slices from WT and double Ras-GRF knockout mice (*grf1/grf2*$^{-/-}$) housed either conventionally (conv) or in EE. The recorded fEPSP slope is expressed as percent of baseline ± SEM, and data were pooled from each group (black boxes, wild-type slices, n = 8; red circles, *grf1/grf2*$^{-/-}$ (conv) slices, n = 9; green triangles *grf1/grf2*$^{-/-}$ (EE, n = 6).

(B and C) 6-month-old wild-type (B) or Ras-GRF knockout mice (C) were exposed to a conventional environment (Conv) or an enriched environment (EE) for 2 weeks. Some mice were exposed to EE only once for 2 hr right before sacrifice (Novel). Hippocampal slices were exposed to theta burst stimulation as described in Figure 1. The recorded fEPSP slope is expressed as percent of baseline ± SEM, and data were pooled from each group.

(B) Conv, black boxes, n = 6 slices/4 mice; EE, red circles, n = 8 slices/5 mice; Novel, green triangle, n = 7 slices/4 mice.

(C) Conv, black boxes, n = 9 slices/6 mice; EE, red circles, n = 7 slices/5 mice; Novel, green triangle, n = 6 slices/4 mice.

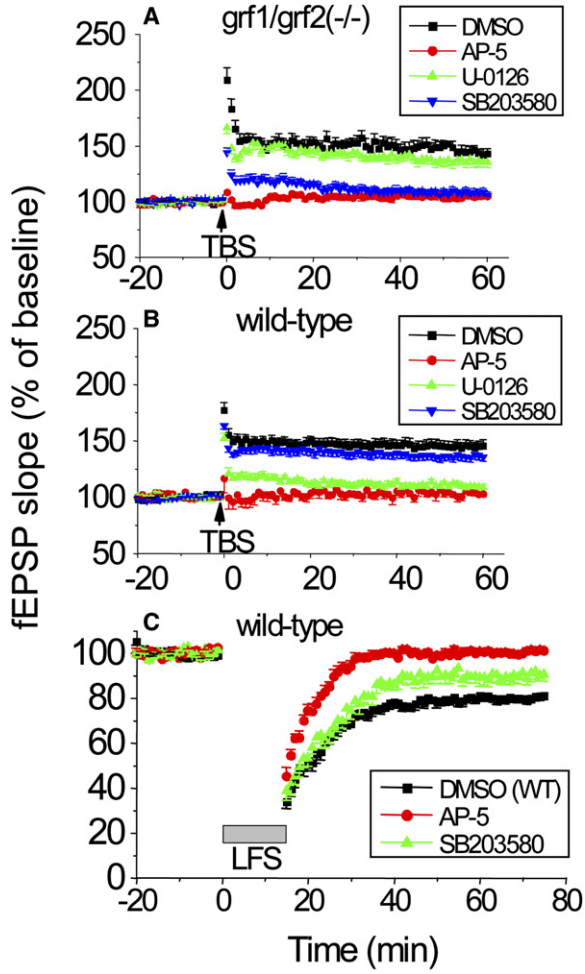

Figure 3. Enriched Environment Promotes LTP through a p38-Dependent Signaling Pathway

(A) Hippocampal brain slices from ~1-month-old double Ras-GRF (*grf1/grf2*$^{-/-}$) knockout mice exposed to an enriched environment were pretreated with buffer containing DMSO (black boxes, n = 7 slices/5 mice), the NMDAR inhibitor AP-5 (100 μM, red circles, n = 5 slices/3 mice), the MEK inhibitor U0126 (20 μM, green triangle, n = 7 slices/5 mice), or the p38 MAPK inhibitor SB203580 (5 μM, blue triangle, n = 9 slices/7 mice) before TBS stimulation. Data were expressed as in Figure 1.

(B) Hippocampal brain slices from 1-month-old WT mice housed conventionally (Conv) were treated as in (A) (DMSO, n = 16; AP-5, n = 4; U0126, n = 7; and SB203580, n = 12).

(C) Hippocampal brain slices from 1-month-old WT were exposed to low-frequency stimulation (LFS) (900 pulses at 1 Hz) to induce LTD after pretreatment with either DMSO (black boxes), SB203580 (green triangles), or AP-5 (red circles). Data are expressed as in Figure 2.

a single exposure to EE just before analysis (Novel). EE induced a modest increase in the magnitude of LTP compared to that found in conventionally housed mice (158.8% ± 5.4% versus 145.6% ± 4.9%, $p < .05$), while a single exposure to EE induced little, if any, effect. No effect of EE on basal synaptic transmission was observed (see Figure S1 in the Supplemental Data available online). As we showed previously [41], hippocampal slices from 1-month-old double Ras-GRF knockout mice housed in a conventional lab environment displayed a clear decrease in theta burst-induced LTP compared to that found in similarly housed WT mice (114.3% ± 3.2% versus 145.6% ± 4.9% $p < .01$; Figure 1B). Strikingly, LTP induction was restored to almost the same level as that found in samples from wild-type animals (148.3% ± 9.5% versus 145.6% ± 4.9%, $p > .05$; compare red circles in Figure 1B to those in Figure 1A) in brain slices from mutant mice exposed previously to 14 days of EE. In contrast, samples from mice given a single brief exposure to EE (Novel) retained the LTP defect (111.9% ± 3.4%; Figure 1B, green triangles).

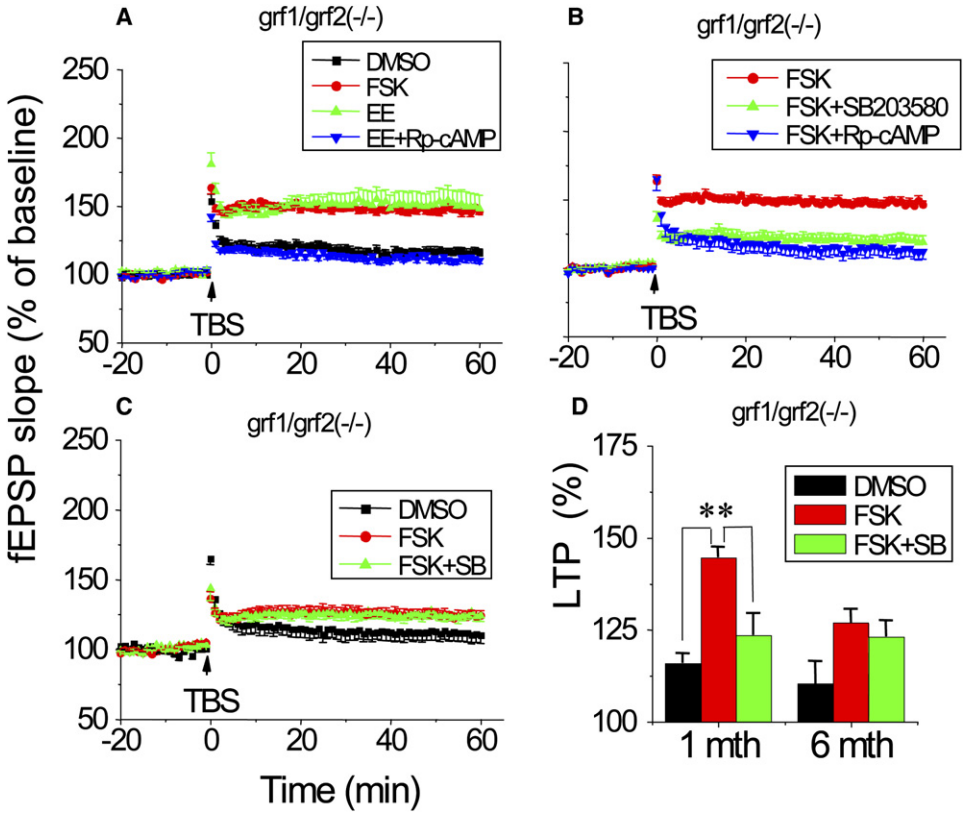

Figure 4. Enhanced cAMP Signaling Can Mimic Enriched Environment by Promoting LTP through a p38-Dependent Mechanism in Samples from Young but Not Mature Mice

(A) Hippocampal slices from 1-month-old *grf1/grf2*$^{-/-}$ mice were exposed to DMSO (black boxes, n = 15), forskolin (25 μM, red circles, n = 10). Slices from mutant mice were also exposed to EE and treated with buffer (green triangles, n = 9) or Rp-cAMPS (100 μM, blue triangles, n = 7).

(B) Brain slices from ~1-month-old *grf1/grf2*$^{-/-}$ mice were treated with forskolin (red circles, n = 11), forskolin plus Rp-cAMPS (blue triangles, n = 7), or forskolin plus SB203580 (green triangles, n = 8). The recorded fEPSP slope is expressed as percent of baseline ± SEM, and data were pooled from each group.

(C) Hippocampal slices from 6-month-old *grf1/grf2*$^{-/-}$ mice were treated with forskolin (red circles, n = 9) or forskolin plus SB203580 (green triangles, n = 7).

(D) Bar graph comparing LTP stimulation of samples from 1-month-old and 6-month-old *grf1/grf2* mice exposed to forskolin or forskolin plus SB203580.

Similar results were obtained when another LTP-inducing protocol, two trains of high frequency stimulation (HFS), was used (Figure S2). Thus, as much as theta burst and HFS-induced LTP represent changes in synaptic plasticity, EE is able to mask at least one genetic defect in synaptic plasticity displayed in Ras-GRF knockout mice housed conventionally.

An Enriched Environment Fails to Rescue Impaired LTD in Adolescent Mice or LTP in Adult Ras-GRF Knockout Mice

This effect of EE on synaptic plasticity was quite specific because it failed to rescue the LTD defect found in these double Ras-GRF knockout mice (Figure 2A). Thus, low-frequency stimulation (LFS) induced a decrease in synaptic activity that was maintained for at least 80 min in samples from wild-type mice, but quickly returned to normal in samples from mutant mice housed either conventionally or exposed to EE.

Moreover, EE rescue of the LTP defect was age dependent. Thus, in 6-month-old wild-type mice, EE produced a small increase in the magnitude of theta burst LTP in wild-type animals (Figure 2B). However, it did not significantly rescue the LTP defect present in 6-month-old Ras-GRF knockout mice (119.5% ± 6.1% versus 115.9% ± 3.9%, $p > .05$; Figure 2C) as it did in 1-month-old mutant mice. Thus, limited exposure to an enriched environment rescues LTP but not LTD defects in 1-month-old mutant mice. However, this environmental effect is lost upon maturation to adulthood.

An Enriched Environment Promotes LTP through a p38-Dependent Signaling Pathway

To begin to understand how EE exerts its effects, the signaling pathways involved in rescuing the LTP defect in Ras-GRF knockout mice were investigated. AP-5, an inhibitor of NMDARs, was found to block rescue of theta burst-induced LTP after exposure to EE in 1-month-old Ras-GRF knockout mice (Figure 3A, red circles), just as it did in WT mice housed conventionally (Figure 3B, red circles). Thus, LTP induced in samples from mice exposed to EE or a conventional environment involves NMDARs.

We showed recently that the suppression of LTP in Ras-GRF knockout mice is due, at least in part, to the

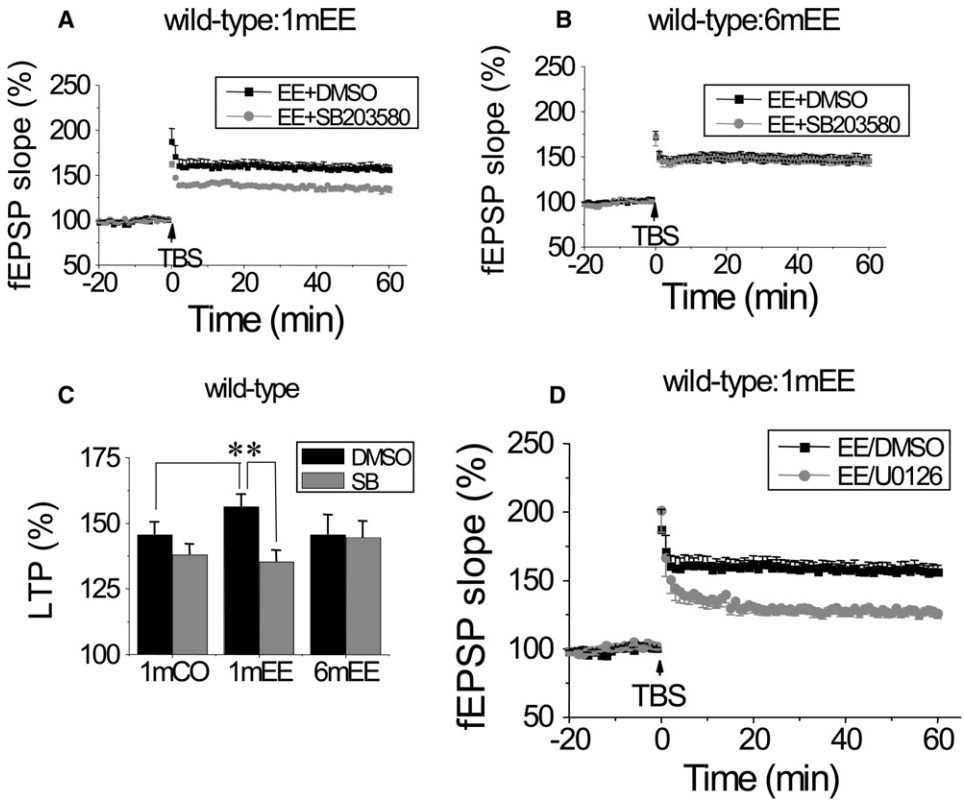

Figure 5. Enriched Environment Generates a p38 Map Kinase-Dependent Component of LTP in Young but Not Mature Wild-Type Mice

(A) The TBS-induced LTP in DMSO-treated (black boxes, n = 8 slices/5 mice) or SB203580-treated (red circles, n = 8 slices/5 mice) brain slices from 1-month-old wild-type mice exposed to EE.

(B) The TBS-induced LTP in DMSO-treated (black boxes, n = 8 slices/6 mice) or SB203580-treated (red circles, n = 8 slices/5 mice) brain slices from 6-month-old wild-type mice exposed to EE.

(C) Bar graph comparing LTP stimulation of DMSO-treated or SB203580-treated brain slices from Conv-exposed or EE-exposed 1-month-old and 6-month-old WT mice.

(D) The TBS-induced LTP in DMSO-treated (boxes, n = 8 slices/5 mice) or U0126-treated (circles, n = 7 slices/4 mice) 1-month-old wild-type mice exposed to EE. Statistically significant differences ($p < .05$) in the bar graph are starred (**).

loss of NMDAR activation of Erk MAP kinase [41]. Erk MAP kinase can be activated by many distinct signaling pathways, so we tested the possibility that EE rescued LTP through a Ras-GRF-independent, Erk activation mechanism. Remarkably, U-0126, an inhibitor that prevents Erk activation, did not block EE rescue of LTP in samples from mutant mice (135.9% ± 5.1%; Figure 3A, green triangles), even though it did, as expected, suppress LTP induction in samples from wild-type mice housed conventionally (109.0% ± 2.8%; Figure 3B, green triangles). These findings imply that EE promotes an NMDAR-mediated, LTP-inducing signaling pathway that is distinct from that used by Ras-GRF proteins.

Even more striking was the observation that treatment with the p38 MAP kinase inhibitor, SB203580, blocked rescue of theta burst LTP by EE from GRF knockout mice (105.9% ± 3.1%; Figure 3A, blue triangles). This finding was a surprise because it has been shown previously [30] and here in Figure 3B (blue triangles) that this inhibitor has little, if any, effect on the induction of LTP in the CA1 region of the hippocampus of WT mice housed conventionally (137.4% ± 4.1% versus 145.3% ± 4.9%, $p > .05$). Instead, the p38 inhibitor suppresses the induction of LTD [29, 30] (see Figure 3C).

An Enriched Environment Functions through a cAMP-Dependent Mechanism

Previous studies have observed an increase in the contribution of cAMP signaling to LTP induction in CA1 region of the hippocampus after long-term exposure to EE [43]. Thus, we tested whether cAMP signaling could be involved in this newly identified p38 pathway to LTP. In fact, pretreatment of brain slices of Ras-GRF knockout mice with the cAMP analog, Rp-cAMPS, blocked EE rescue of LTP in Ras-GRF knockout mice (110.3% ± 2.5%; Figure 4A, blue triangles) at least as effectively as the p38 inhibitor (see Figure 3A, blue triangles), indicating that cAMP and p38 are both involved in EE action. Based on this finding, forskolin, an activator of adenylate cyclase, was tested for its ability to mimic EE in rescuing theta burst LTP in Ras-GRF knockout mice. Figure 4A (red circles) shows that forskolin did, in fact, rescue theta burst-induced LTP induction in hippocampal slices from young Ras-GRF knockout mice housed conventionally, to a degree comparable to that observed after exposure to EE (Figure 4A, green triangles). Importantly, forskolin rescue of LTP was blocked by the p38 inhibitor SB203580 (118.9% ± 6.0%; Figure 4B, green triangles) to a level almost the same as that observed by the addition of the cAMP analog,

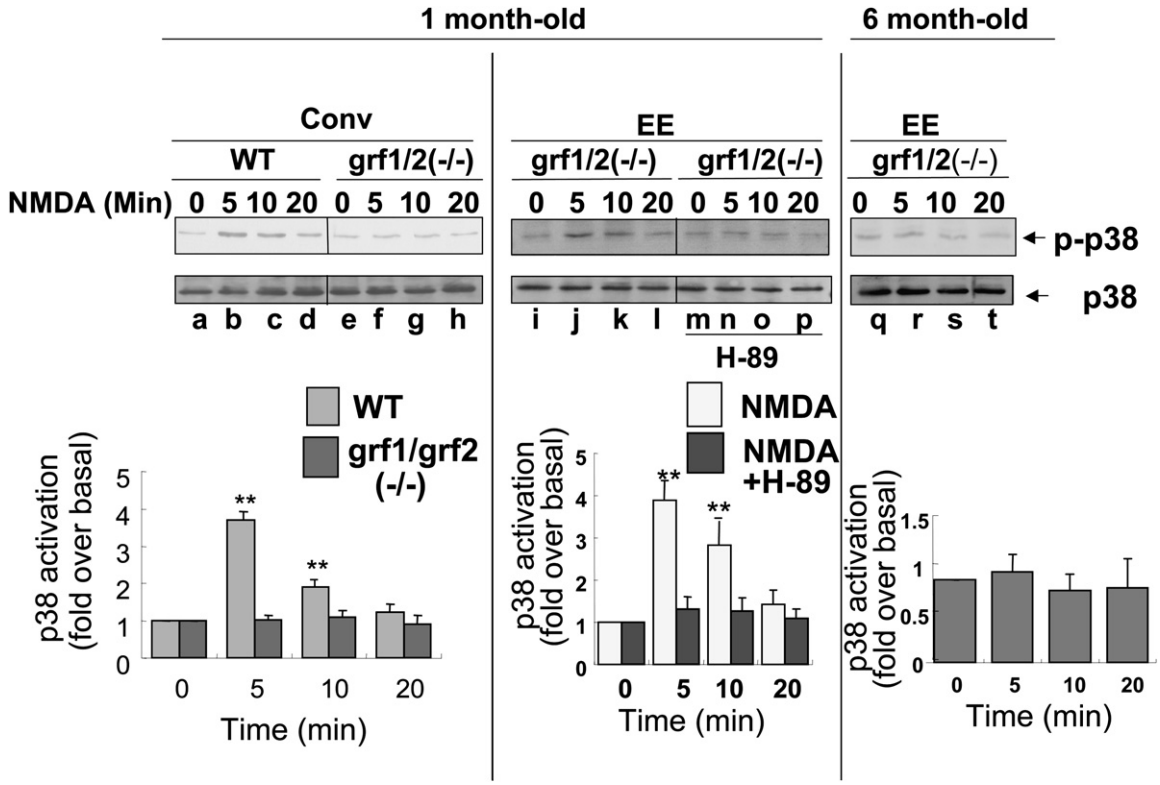

Figure 6. Enriched Environment Enables p38 Activation by NMDA Receptors through a cAMP-Dependent Gating Mechanism

1-month-old (lanes a–p) or 6-month-old (lanes q–t) WT (lanes a–d) or double Ras-GRF knockout (lanes e–t) mice were exposed to a conventional environment (lanes a–h) or enriched environment (lanes i–t). Brain slices from these mice were treated with NMDA for various lengths of time, and p-p38 or total p38 proteins were detected in lysates by immunoblotting. In some cases, the brain slices were pretreated the PKA inhibitor, H-89 (5 μM) (m–p). Bar graphs represent the averaging (±SEM) of data from at least three experiments. Statistically significant differences are starred (**p < .01).

Rp-cAMPS (112.1% ± 5.6%; Figure 4D, blue triangles), implying that cAMP functions, at least in part, through p38 activation to contribute to EE-facilitated LTP in Ras-GRF knockout mice.

Finally, forskolin was less effective in rescuing defective LTP in 6-month-old than 1-month-old Ras-GRF knockout mice (124.2% ± 3.4% versus 144.6% ± 2.9%, p < .05; compare Figures 4A and 4C, red circles, and red bars in graph in Figure 4D). Importantly, the small rescue of the LTP defect by forskolin in 6-month-old mice was not blocked by the p38 inhibitor (124.1% ± 3.9%; Figures 4C and green bars in Figure 4D). This implies that this LTP induction is through an alternative mechanism that is not necessarily related to EE. Thus, the observed lack of effectiveness of EE in mature knockout mice is due, at least in part, to an age-induced loss of cAMP signaling potential.

An Enriched Environment Generates a p38 Map Kinase-Dependent Component of LTP in Adolescent but Not Adult Wild-Type Mice

To confirm that EE induces a p38 component to LTP induction in wild-type mice, the sensitivity of theta burst-induced LTP to the p38 inhibitor SB203580 was tested in these mice after exposure to EE. As shown previously [30], we find here that p38 inhibition has no statistically significant suppressive effect on LTP induction in samples from mice exposed to a conventional environment (Figures 3B and 5C). In contrast, p38 inhibition suppressed the theta burst-induced increase in synaptic transmission in samples from young mice exposed to EE by ~38% (156.3% ± 4.8% versus 135.3% ± 4.3%, p < .01; Figures 5A and 5C), an amount comparable to the role of Erk MAP kinase in samples from mice housed conventionally. Moreover, SB203580 produced no significant inhibitory effect in samples from 6-month-old wild-type mice exposed to EE (146.3% ± 6.3% versus 146.7% ± 6.9%; Figures 5B and 5C).

LTP induction in wild-type mice exposed to EE still depended upon Erk signaling, as shown by the fact that its magnitude was suppressed ~45% by the inhibitor of the Erk signaling cascade U-0126 (Figure 5D). Thus, LTP induction in 1-month-old mice exposed to this form of EE involves an additional previously unidentified signaling pathway that is mediated, at least in part, by p38 activation. However, this component of theta burst-induced LTP induction is lost by 6 months of age.

An Enriched Environment Enables p38 Activation by NMDA Receptors through a cAMP-Dependent Gating Mechanism in Adolescent but Not Adult Ras-GRF Knockout Mice

To assess directly the effect of EE and age on specific signaling molecules implicated in the electrophysiology experiments described above, signaling through NMDARs was assayed in hippocampal slices from mice housed

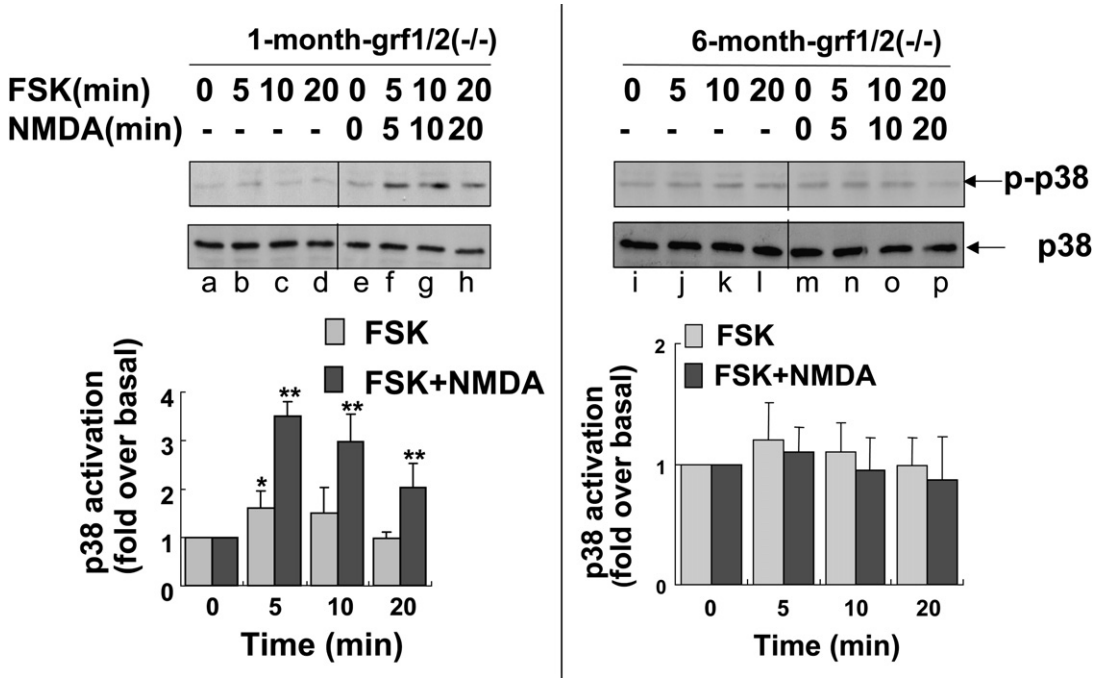

Figure 7. Forskolin and NMDA Receptors Cooperate to Activate p38 Map Kinase in the Hippocampus of Young but not Mature Mice

Brain slices from 1-month-old (lanes a–h) or 6-month-old (lanes i–p) double Ras-GRF knockout mice were treated with either forskolin (25 μM) (lanes a–h, i–p) or forskolin plus NMDA (lanes e–h, m–p) for various lengths of time. Lysates were prepared, and p-p38 or total p38 proteins were detected as described above. Bar graphs represent the averaging (±SEM) of data from at least three experiments. Statistically significant differences are starred (*p < .05; **p < .01).

conventionally or after exposure to EE. We showed recently [41] and here (Figure 6, lanes a–h) that NMDA activation of p38, assessed by immunoblotting hippocampal slices with activation-specific p38 antibodies, is lost in brain slices from double Ras-GRF1/Ras-GRF2 knockout mice (*grf1*/*grf2*) housed conventionally (Conv). This is because Ras-GRF1 mediates NMDAR activation of p38 to promote LTD [41]. In striking contrast, samples from double Ras-GRF knockout mice exposed to EE displayed ~3-fold activation of p38 after stimulation with NMDA (Figure 6, lanes i–l and bar graph). Furthermore, NMDA activation of p38 was blocked by the PKA inhibitor H-89 (Figure 6, lanes m–p and bar graph). Moreover, EE did not rescue NMDA activation of Erk (Figure S3). Finally, exposure of 6-month-old mice to EE failed to positively gate NMDAR activation of p38 MAP kinase (Figure 6, lanes q–t and bar graph). Overall, these biochemical experiments are consistent with and add to our electrophysiology experiments by showing that in young but not mature mice, EE enables NMDARs to activate p38 through a cAMP-dependent mechanism.

Because our electrophysiology experiments showed that forskolin mimics EE in rescuing the LTP defect in Ras-GRF knockout mice (see Figure 4A), we tested the ability of forskolin to activate p38 in hippocampal slices from 1-month-old mutant mice housed conventionally. Forskolin added alone did not significantly activate p38 (Figure 7, lanes a–d, and bar graph), and neither did NMDA (see Figure 6, lanes e–h and bar graph). However, when the two agents were added together to mimic the rescue of LTP by theta burst stimulation performed in the presence of forskolin (see Figure 4A), p38 activation was observed (Figure 7, lanes e–h and bar graph). These findings are consistent with a model whereby EE facilitates NMDAR activation of p38 through a cAMP gating mechanism in the hippocampus of young mice.

Finally, consistent with previous experiments in this study, no p38 activation was observed when 6-month-old Ras-GRF knockout mice were treated similarly (Figure 7, lanes i–p, and bar graph), indicating that the age-dependent loss in EE action is due at least in part to the failure of NMDAR activation plus cAMP signaling to activate p38 Map kinase in the adult hippocampus.

An Enriched Environment Enables p38 Activation by an NMDA Receptor and cAMP-Dependent Gating Mechanism in Adolescent but Not Adult WT Mice

In contrast to Ras-GRF knockout mice, wild-type mice have an intact NMDAR/Ras-GRF1/p38 signaling cascade that is thought to promote LTD. This makes specific detection of an EE-induced NMDAR/p38 signaling cascade that promotes LTP more difficult to detect. Recent studies have suggested that LTP induction occurs primarily through synaptic NMDAR signaling [44, 45], which can be preferentially activated by stimulation of tissue with bicuculline, a GABA receptor inhibitor that removes inhibitory action of GABA and thus induces an increase in glutamate-stimulated activity [44, 46]. Thus, we tested bicuculline activation of p38 in hippocampal slices from wild-type mice and found that it activated p38 only after exposure of mice to EE (Figure 8A). Just as we found in Ras-GRF knockout mice, this EE-enabled signaling pathway was dependent upon NMDARs, since it was blocked by APV (Figure 8B). It was also dependent upon cAMP signaling, since it was also blocked by the PKA inhibitor, H89 (Figure 8B). Moreover,

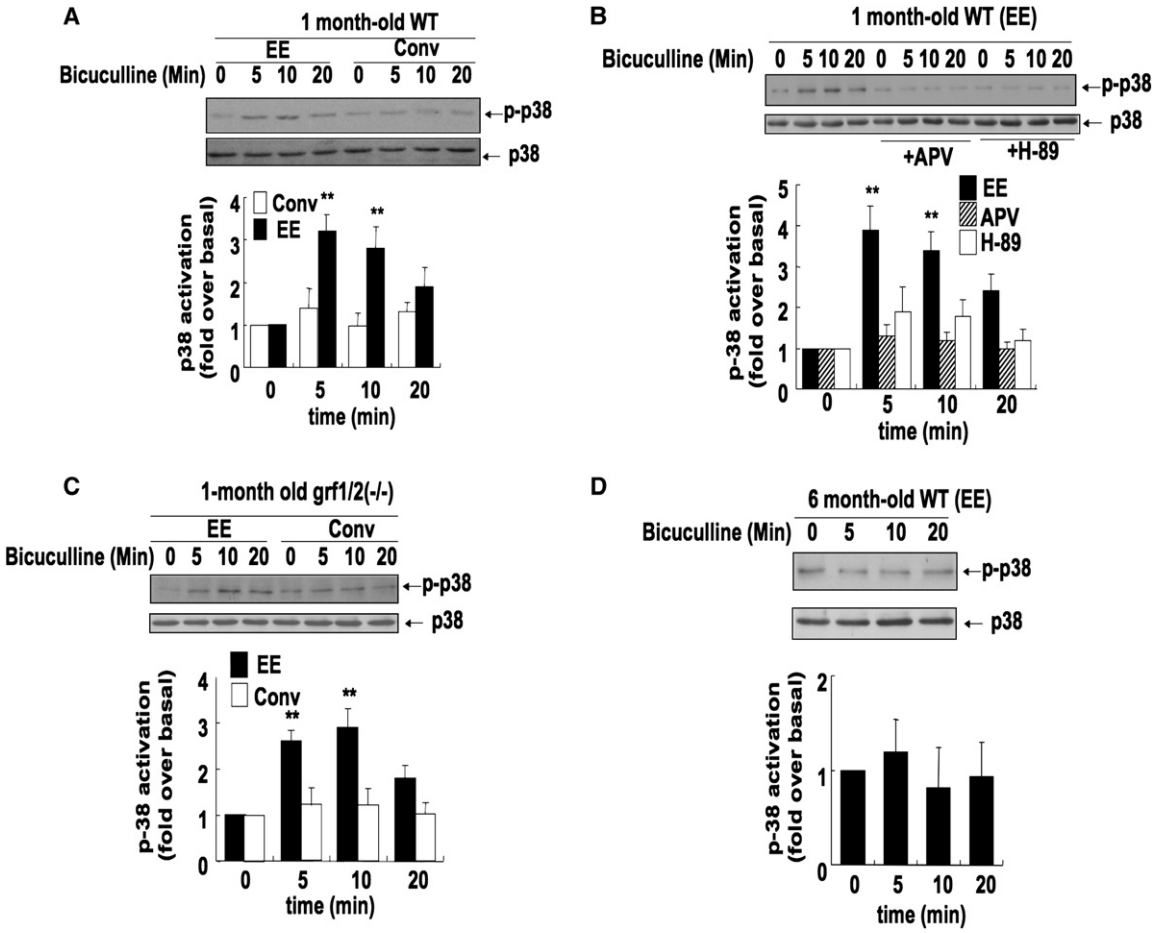

Figure 8. Bicuculline Activates p38 Only after Exposure of Adolescent but Not Adult WT Mice to EE

Brain slices from 1-month-old (A–C) WT or Ras-GRF knockout mice and 6-month-old WT mice (D), exposed to either a conventional environment or EE, were stimulated with bicuculline (10 μM) for various amounts of time, and p-p38 or total p38 levels were assessed in lysates. Some samples were preincubated with inhibitors as indicated. Bar graphs represent the averaging (±SEM) of data from at least three experiments. Statistically significant differences are starred (**p < .01).

consistent with this being an LTP-inducing rather than an LTD-inducing p38 activation, EE unlocked p38 activation by bicuculline even in slices from Ras-GRF knockout mice, where the NMDAR/p38 pathway to LTD is eliminated (Figure 8C). Finally, as would be predicted from our electrophysiology data on WT mice (Figure 5), EE did not allow bicuculline to activate p38 in 6-month-old mice exposed to EE (Figure 8D).

Discussion

Overall, this study adds a significant new dimension to our understanding of cell-signaling regulation by demonstrating that the degree of environmental enrichment to which an animal is exposed gates the flow through a specific signaling pathway in the hippocampus of young mice. Importantly, this signaling pathway represents a newly identified component of LTP induction that is functional in the hippocampus of adolescent mice exposed to an enriched environment but nonfunctional in mice housed conventionally. Finally, the influence of the environment on this signaling pathway and on its ability to influence LTP induction is developmentally regulated (see model in Figure 9).

This newly identified signaling cascade, which we identified first by its ability to rescue defective LTP in adolescent Ras-GRF knockout mice and then as an newly detected component of LTP induction in adolescent wild-type mice, connects NMDA glutamate receptors (NMDARs) to p38 MAP kinase activity. This signaling pathway is enabled in the CA1 region of the hippocampus of young mice after exposure to a 2 week period to an enriched environment (EE) (Figure 9A), which consists of exposure to a changing complex environment as well as opportunities for voluntary exercise, both of which have been identified as mediators of alterations in brain function [1]. In contrast, this signaling cascade is blocked and thus insensitive to activated NMDARs in the hippocampus of mice exposed to a typical, "deprived" animal cage environment containing only plain bedding, food, and water (Figure 9B). The EE-mediated gating mechanism underlying this phenomenon involves cAMP signaling, since a cAMP analog blocked EE-induced LTP and EE facilitation of NMDAR/p38 signaling. In addition, forskolin, an activator of adenylyl cyclase, mimicked EE by both rescuing defective LTP in Ras-GRF knockout mice and by cooperating with NMDARs to promote p38 activation in young mice

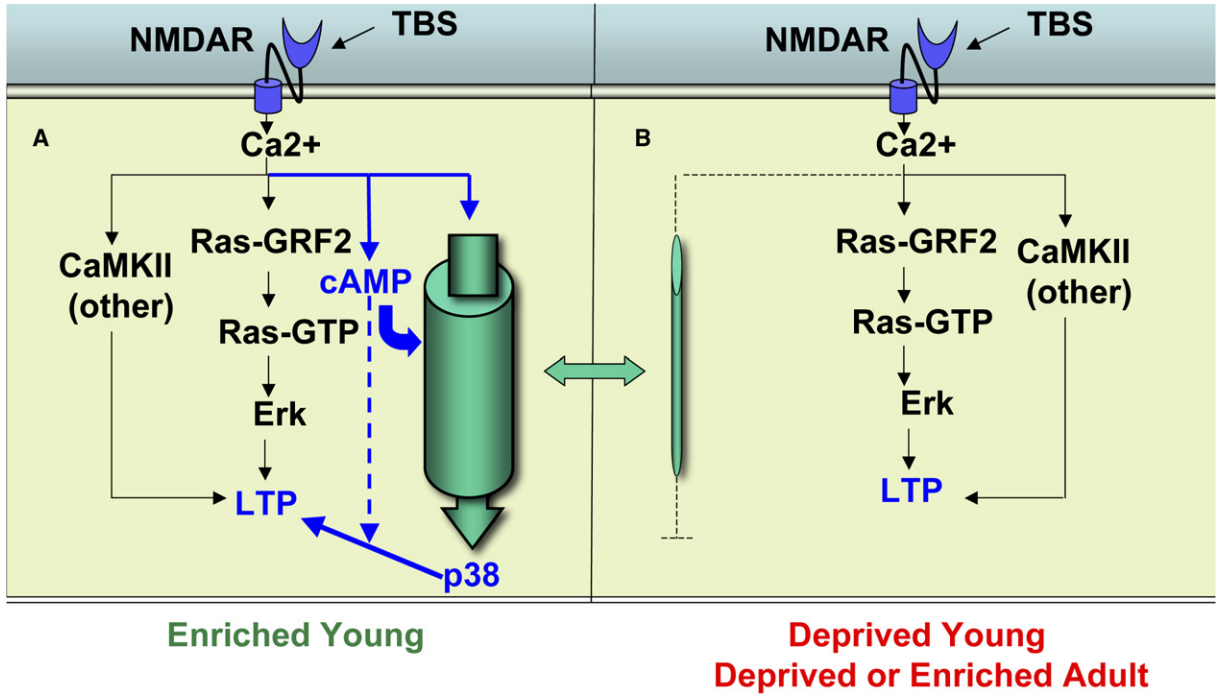

Figure 9. Environmental Control of a Novel Signaling Pathway in Young but Not Mature Mice that Induces LTP

(A) In young mice (1-month-old), an enriched environment (EE) enables NMDA glutamate receptors (NMDARs) to activate p38 MAP kinase through a cAMP-dependent gating mechanism in response to theta burst (TBS) stimulation. Activated p38, and possibly cAMP through a p38-independent mechanism (dotted line), contribute to NMDA receptor-mediated LTP induction in the CA1 region of the hippocampus, along with Erk Map kinase activated by Ras-GRF2, calmodulin-dependent kinase II (CaMKII) (NMDAR activation of ErK is CaMKII independent in hippocampal neurons; [50] and X.T. and L.A.F., unpublished observation) and other calcium-responsive signaling pathways. This p38 pathway could function postsynaptically, presynaptically at this synapse, or indirectly in cells that modulate synaptic function like interneurons. In mice lacking a functional Ras-GRF2, where NMDA activation of ERK does not occur, this environmentally enabled cAMP/p38 pathway restores NMDAR-mediated LTP to almost wild-type levels.

(B) In young mice exposed to a conventional lab environment (which may be considered "deprived"), NMDARs are no longer able to activate p38 through this pathway (they still activate p38 through Ras-GRF1 to promote LTD [41]). Similarly, NMDAR activation of p38 through this pathway does not occur in mature mice (6 months old) even if mice are exposed to a comparable EE at this age.

housed conventionally. The involvement of cAMP in this gating mechanism is consistent with an earlier finding that the contribution of cAMP signaling to LTP induction in the CA1 region of the hippocampus is enhanced by long-term housing in enriched cages [43] and with previous studies highlighting cAMP as an effective gating molecule as assessed in cell-culture systems [47].

Interestingly, EE-induced p38 signaling did not rescue defective NMDAR-dependent LTD in Ras-GRF knockout mice even though the LTD defect in these mutant mice is thought to be due, at least in part, to a loss of NMDAR-activated p38 [41]. Thus, the p38 activated by this EE-enabled signaling pathway is specific for LTP rather than LTD. The most likely mechanism underlying this difference is that in a conventional environment NMDARs activate only the subset of p38 substrates and signaling pathways that specifically promote LTD, whereas after exposure to EE, NMDARs activate an additional, distinct subset of p38 substrates and signaling cascades that specifically promote LTP.

Although EE is described as an experimental variable in this and other studies, it is most likely more representative of a natural environment for a mouse than is standard laboratory housing. Therefore, if the inbred strains we used are representative of mice in general, the p38-mediated signaling pathway we have identified after EE exposure is likely a normal component of NMDAR induction of LTP in young animals living in the wild. This component of LTP induction has not been detected previously most likely because key experiments demonstrating that Erk regulates LTP and p38 regulates LTD were performed on samples from young animals housed conventionally [30, 48, 49]. Thus, hippocampal slices from these mice show no significant defect in LTP induction after exposure to a p38 MAP kinase inhibitor (see Figure 3 and [30]), whereas we find that LTP is inhibited ~38% in samples from wild-type mice exposed to EE. Presumably, this p38-mediated pathway cooperates with other LTP-inducing mechanisms that have already been detected in samples from mice housed conventionally, including Ras-GRF2-mediated Erk activation, CaMKII activity (NMDARs activate Erk through a CaMKII-independent mechanism in hippocampal neurons; [50] and X.T. and L.A.F., unpublished data), and other mechanisms, to promote LTP in mice living in an enriched environment (see Figure 9).

Recent studies on NMDAR-mediated LTP mechanisms in the CA1 region of the hippocampus of conventionally housed mice have highlighted the importance of enhanced delivery of AMPA receptors to the

postsynaptic membrane [23]. Presynaptic mechanisms of LTP have also been demonstrated at this [24] and other [22] synapses. This newly identified p38 Map kinase-signaling pathway could function by either of these mechanisms or indirectly through modulating cells such as interneurons. It also remains to be determined whether p38 can promote LTP at other sites as well.

Another key finding of this study is that while this EE protocol can positively gate this p38 signaling pathway and promote LTP in 1-month-old mice, it loses this capability in both mutant and wild-type adult mice (6 months old). This loss of effectiveness is associated with the inability of cAMP signaling to promote NMDAR-mediated p38 activation. As such, this EE can no longer rescue defective LTP in 6-month-old Ras-GRF knockout mice. Moreover, there is no longer a suppressive effect of a p38 inhibitor on LTP induction in mature EE-exposed wild-type mice, as there is in their 1-month-old counterparts. Revealing how LTP contributes differently to behavior in the adult when the induction of the p38 component to LTP by the environment is suppressed may uncover new insights into the physiological significance of the decline in brain plasticity upon aging.

In young Ras-GRF knockout mice, the loss of the NMDAR/Ras-GRF2/Erk MAP kinase pathway is compensated by this EE-facilitated, NMDAR/cAMP/p38 MAP kinase-signaling pathway. Polymorphic alleles that weaken the function of *ras-grf2* or other genes encoding proteins involved in LTP induction are likely to be prevalent in the human population. Our studies suggest that some behavioral defects in individuals with these flawed molecules may be suppressed in the young by this novel EE-gated signaling pathway, as long as their environment is enriched. However, such defects might emerge if they live in a "deprived" environment in their youth and continue to be expressed even if they are exposed to EE as adults.

Finally, gating of specific signaling cascades by the level of environmental enrichment is likely to be quite common in the brain and may even occur in other tissues. Thus, a thorough understanding of environmental control over specific cell-signaling cascades may be needed to most effectively target signaling molecules for the treatment of human diseases.

Supplemental Data

Supplemental Data include three figures and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/23/2303/DC1/.

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